

Virus Transfer at the Skin–Liquid Interface

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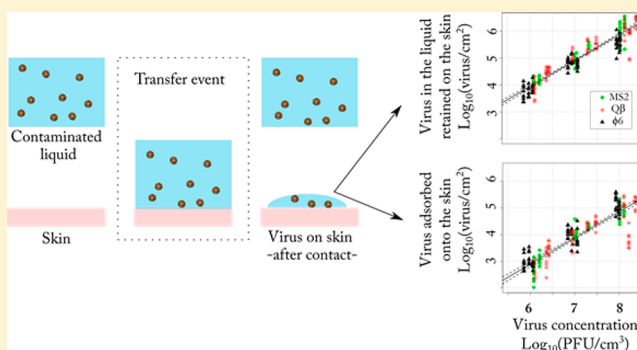
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Supporting Information

ABSTRACT: Understanding virus transfer between liquid and skin is necessary to estimate transmission during water-related activities. Here, we modeled virus transfer from liquid-to-skin and skin-to-liquid. We performed human subject studies using three bacteriophages as pathogenic virus surrogates: nonenveloped MS2 and Q β and enveloped Φ 6. Our study shows that transfer from liquid-to-skin is describable by a single model based on (1) virus concentration and (2) volume of liquid remaining on skin. Contact times (0.1–30 min), and virus species had little-to-no influence on virus transfer. Likewise, liquid conditions (pH 6–9, ionic strength 10–550 mM) had no influence on transfer as shown for MS2. The model accounts for both, virus adsorbed onto the skin, and virus in the liquid retained on skin. In comparison, virus transfer from skin-to-liquid was influenced by the wetness of the skin and by liquid type (water, saliva). $90 \pm 19\%$ of the virus inoculated on the skin are transferred to the water when the skin remains wet compared to $30 \pm 17\%$ when the skin is dry. The transfer from skin-to-liquid was 41% higher when the recipient liquid was water as compared with saliva. This study quantifies virus transfer between liquid and skin and guides risk assessments of water-related activities.



■ INTRODUCTION

Enteric viruses are one of the leading causes of diarrhea^{1,2} and are responsible for a significant portion of drinking and recreational water outbreaks.^{3–5} Waterborne enteric viruses are mainly transmitted via the fecal–oral route,⁶ including both direct ingestion of contaminated water (inclusive of both accidental and intentional ingestion) and indirect transfer (water-to-hand and hand-to-mouth). To date, risk assessments of waterborne pathogens have largely focused only on direct ingestion.^{7–9} The estimated ingested dose for direct ingestion is calculated based on assumptions of the volume of liquid ingested and concentration of pathogens in the liquid.¹⁰ Example scenarios include risk assessments for drinking water,^{11,12} swimming,^{7,13} fishing, and canoeing.¹⁴ In many scenarios neglecting contributions from indirect transmission of pathogens likely underestimates risks, especially where indirect ingestion is more likely than direct ingestion.

In studies that consider the indirect transfer of microorganisms due to water contacts, indirect transfer is estimated based on either estimating volume of the liquid transferred or by performing pathogen transfer experiments.^{15–17} Specifically, De Man et al. estimated total pathogen transfer based on the thickness of the water film that remained on the skin after hand

contacts with water, along with information on the pathogen concentration in the water.¹⁵ In contrast, O’Toole et al. determined experimentally the percentage of bacteria and virus transferred to the hand after contact with contaminated liquid using one specific volume and one specific concentration of pathogens in the water. These studies assume that system properties (such as characteristics of the virus, liquid, and skin) do not influence transfer. However, from the more extensive literature on virus transfer from objects to hands, these factors are known to influence transfer.^{18–23}

Understanding virus transfer between liquid and skin is necessary to estimate indirect transmission during water-related activities. Although there is little data available on transfer between liquids and skin, parallels may be observed in the literature of virus transfer between liquids and surfaces, and between surfaces and skin. Viruses are readily transferred from liquid to a variety of surfaces including food,^{24,25} soils,^{26,27} minerals,²⁶ sand,²⁸ silica,^{24–31} and organic matter.^{24–31} The

Received: September 23, 2017

Revised: November 15, 2017

Accepted: November 21, 2017

Published: November 21, 2017

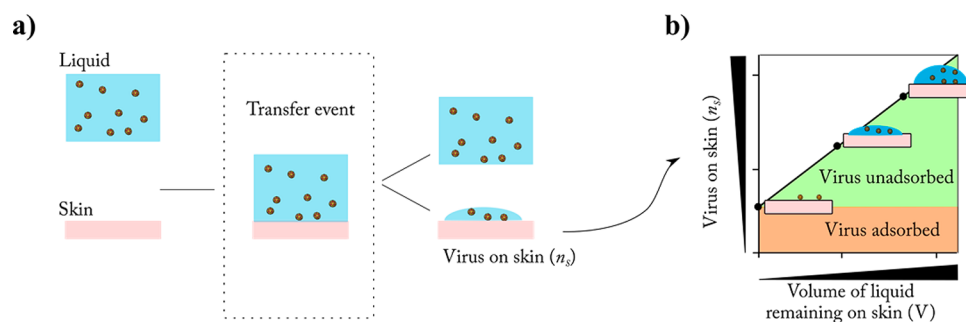


Figure 1. Conceptual model of liquid-to-skin transfer. (a) After skin–liquid contact (transfer event), viruses in the liquid are transferred to the skin (n_s), including adsorbed and unadsorbed fractions. (b) The total number of viruses transferred to the skin, n_s , is equal to the number of viruses adsorbed onto the skin during the contact event plus the unadsorbed viruses in a volume of liquid remaining on the skin (V).

forces driving virus adsorption to surfaces have been described as a complex combination of electrostatic, hydrophobic and van der Waals interactions.^{30,32,33} Electrostatic forces are modulated by virus and surface characteristics and these characteristics, in a liquid environment, are influenced by liquid properties such as pH and ionic strength.^{32,34} This is consistent with evidence suggesting that virus adsorption to surfaces is influenced by virus,^{24,27,30–32} liquid,^{26,28–32,35} and surface characteristics.^{29–31}

Transfer of viruses from surfaces-to-skin has been extensively explored, identifying multiple factors that significantly influence it. These factors include: virus species,^{18,19} skin characteristics,^{19,22} fomite characteristics (e.g., material porosity),^{20,21,23} direction of transfer (i.e., finger-to-surface, surface-to-finger),^{18,19,22} humidity,²¹ inoculum drying time,^{36,37} pressure,³⁷ and friction.³⁷ In contrast, there is virtually no data for skin-to-liquid transfer. Rusin et al. (2002) estimated that 34% of the bacteriophages present in the skin are transferred to the moist environment of lips.²⁰ Nevertheless, no measure of variation was provided in their study, and the saliva-rich environment of the lips will probably not reflect other liquid environments.

The objectives of this work were to quantify virus transfer from liquid-to-skin and skin-to-liquid, identify factors (virus species, liquid characteristics, direction of the transfer, concentration and contact time) that influence virus transfer, and increase the accuracy of risk assessments of human interaction with virus-contaminated liquids. The study provides insight into the dynamics of virus transfer at the skin–liquid interface relevant to the environmental spread of viruses.

■ CONCEPTUAL MODELS

Virus transfer at the liquid–skin interface is a bidirectional phenomenon: viruses are transferred both from liquid-to-skin and from skin-to-liquid. After sufficient contact time between liquid and skin, the system is expected to achieve equilibrium. However, real-world scenarios of skin and liquid contact require different conceptual models to be useful as data inputs in diverse risk assessment frameworks and modeling. Therefore, in the present work, we modeled the transfer from liquid-to-skin differently than the transfer from skin-to-liquid and designed experimental protocols appropriately for each model. In liquid-to-skin transfer, skin is assumed to be in contact with a sufficiently large volume that can be considered infinite relative to the surface area of the skin in contact (i.e., swimming in a lake). Virus transfer will not depend on the absolute number of viruses in the liquid but rather on the concentration of virus in the liquid; the contact time; the surface area of the skin in

contact with the liquid; and the physicochemical properties of the skin, liquid, and virus. In contrast, in skin-to-liquid transfer, the skin surface area is finite (relative to the liquid), so the total amount of virus per skin surface area can be estimated. Therefore, skin-to-liquid transfer can be modeled as the percentage of viruses transferred to the liquid after contact, relative to the initial number of viruses on the skin. The number of viruses on the skin is defined as the concentration of viruses on the skin (viruses/area) times the contact area between the liquid and the skin.

Liquid-to-Skin Model. Here, we assume that viruses adsorb to the skin surface during liquid-to-skin contact. The magnitude of adsorption depends on the properties of the system (skin, virus, and liquid characteristics). We refer to this as the “adsorbed” virus. Additionally, contaminated liquid will be retained on the skin (i.e., film thickness), due to incomplete removal of the liquid. We refer to this as the “unadsorbed” virus. We assume the number of unadsorbed viruses is a function of the volume of liquid remaining on the skin and the concentration of viruses in the liquid (Figure 1, eq 1).

Experimentally, adsorbed viruses must be measured after complete removal of the contaminated liquid. The conceptual model of liquid-to-skin transfer is described as

$$n_s = n_{\text{ads}} + n_{\text{unads}} \quad (1)$$

$$n_{\text{unads}} = CV \quad (2)$$

where n_s is the total number of viruses per unit area on the skin (Plaque-forming units (PFU)/cm²), n_{ads} is the number of adsorbed viruses per unit area (PFU/cm²) and n_{unads} is the number of unadsorbed viruses per unit area (PFU/cm²) (eq 1). n_{unads} can be estimated using concentration of viruses in the liquid, C (PFU/mL), and the volume of liquid retained per unit area on the skin, V (ml/cm²) (eq 2). Inactivation is not accounted for in the model because no significant inactivation of the viruses in the liquid is expected to happen on the time scale of the transfer event, ≤ 30 min,^{38,39} as supported by the relatively low inactivation rates of bacteriophages $\Phi 6$ (enveloped) and MS2 (nonenveloped) in liquid at 25 °C (approximately 0.044 and 0.020 h^{−1}).³⁹ Additionally, this model assumes no concentration gradient in the bulk liquid results from adsorption of viruses onto the skin. Because the concentration of viruses in the liquid is orders of magnitude higher than the number of viruses adsorbed onto the skin, it is unlikely that adsorption causes a meaningful gradient.

Skin-to-Liquid Model. In scenarios where contaminated skin is in contact with liquid, we hypothesize that a fraction of the viruses on the skin will be transferred to the liquid (Figure

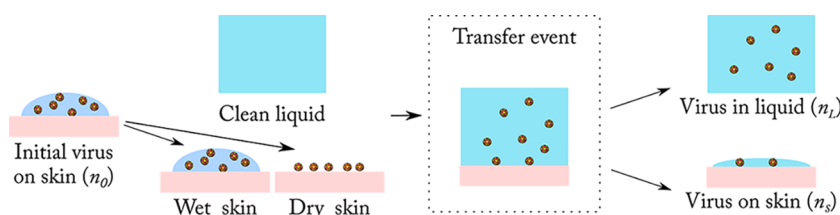


Figure 2. Conceptual model of skin-to-liquid transfer. A known number of viruses initially on the skin (n_0), due for example to contact of the skin with a contaminated liquid, are subsequently transferred from wet contaminated skin or dried contaminated skin to a new clean liquid. After this secondary skin-to-liquid contact (transfer event), a portion of the viruses on the skin will be transferred to the liquid (n_L) and a portion of the viruses will remain on the skin (n_S).

Table 1. Cohort Studies' Description. The Direction of the Transfer, Experimental Design, Treatment Tested, Bacteriophage Used, Number Volunteers, Sessions, And Transfer Events (N) for Every One of the Three Human Subject Studies^a

study	direction	set-up	treatment	phages	volunteers	sessions ^a	N
^b A	liquid → skin	finger immersion	concentration [10^6 , 10^7 , 10^8 PFU/ml] time [0.1, 1, 10, 10 min]	MS2 Qβ Φ6	7	27	210
B	liquid → skin	finger immersion	pH [6, 7.5, 9] ionic strength [10, 550 mM]	MS2	7	7	94
C	skin → liquid	droplet transfer	liquid [saliva, water] drying [wet, dry]	MS2	7	7	56

^aSession = number of times any of the volunteers visited the laboratory to perform a transfer experiment. PFU = plaque-forming units. N = individual transfer event = sample size. ^bIn study A, two volunteers were assigned two different bacteriophages and 5 volunteers were assigned only one bacteriophage. For each bacteriophage assigned, the volunteers visited the laboratory three times (three sessions).

2). The number of viruses transferred to the liquid can be modeled as a skin-to-liquid transfer efficiency (TE_{S-L}). In the present study, we calculate transfer efficiency using two distinct but related estimates: theoretical TE_{S-L} and recoverable TE_{S-L} .

The theoretical TE_{S-L} (eq 3) is defined as the number of viruses recovered in the liquid (n_L) relative to the initial number of viruses on the skin (n_0). This is parallel to equations reported by O'Toole et al.,⁴⁰ and can be used in cases where a known amount of contaminated liquid on the hand is followed by a transfer event. This fraction does not take into account irreversible adsorption of virus onto the skin or virus inactivation.

$$\text{theoretical } TE_{S-L} [\%] = \frac{n_L [\text{PFU}]}{n_0 [\text{PFU}]} \times 100 \quad (3)$$

In contrast, the recoverable TE_{S-L} is defined as the number of viruses recovered in the liquid (n_L), relative to the viruses recovered from the sum of liquid (n_L) and skin (n_S) (eq 4). Virus inactivation is implicit in this equation. This is similar to fomite-mediated transfer equations reported elsewhere,^{19–21} where the transfer efficiency is calculated using only recoverable virus.

$$\text{recoverable } TE_{S-L} [\%] = \frac{n_L [\text{PFU}]}{n_L [\text{PFU}] + n_S [\text{PFU}]} \times 100 \quad (4)$$

In addition to the transfer efficiencies, we calculated the total recovery, which is the total amount of viruses that could be recovered after the transfer event, defined as the sum of viruses recovered in the liquid and skin ($n_L + n_S$), relative to the initial number of viruses on the skin (n_0). A 100% total recovery would imply no loss of viruses during the experiment due to factors such as inactivation or irreversible adsorption.

$$\text{total recovery } [\%] = \frac{n_L [\text{PFU}] + n_S [\text{PFU}]}{n_0 [\text{PFU}]} \times 100 \quad (5)$$

EXPERIMENTAL SECTION

Bacteriophage Production, Purification, and Enumeration. We selected bacteriophages MS2, Qβ, and Φ6 for this study. Both MS2 and Qβ are similar to enteric viruses of human health concern.⁴¹ Their isoelectric points (IEPs) are 3.9 and 4.9, respectively,³⁰ which are close to the IEPs of Human Adenovirus (4.5).³⁴ Similar to Human Enterovirus, Coxsackie virus, and Poliovirus, both MS2 and Qβ have (+) single-stranded RNA genomes and are nonenveloped. Despite their similarities with each other, MS2 and Qβ have very different adsorption behavior at the solid–water interface due to differences in their surface charge and polarity.³⁰ We selected Φ6 as a surrogate for enveloped viruses. Bacteriophage Φ6 has a lipid envelope, double-stranded RNA genome, and has been used in the past as a surrogate for a variety of enveloped viruses,^{39,42–45} including Ebola.⁴²

Bacteriophage MS2 (DSMZ 13767), Qβ (DSMZ 13768), and Φ6 (DSMZ 21518), together with their respective hosts, *E. coli* (DSMZ 5695) for MS2 and Qβ and *P. syringae* (DSMZ 21482) for Φ6, were purchased from the DSMZ German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). To grow and purify the bacteriophages we used a protocol adapted from Armanious et al.³⁰ Briefly; 1 L of Tryptone Soya Broth (AppliChem) containing log-phase bacteria was inoculated with 100 μL of a 10^{10} Plaque Forming Units (PFU)/mL stock of bacteriophage and incubated overnight. The sample was clarified by centrifugation for 15 min at 4000g. The supernatant was concentrated using an

Amicon Ultra centrifugal filter device (100 kDa; Merk Millipore).

The double agar layer procedure was used to enumerate infective bacteriophages.⁴⁶ Shortly, bacteriophage (MS2, Q β or Φ 6) was combined with host bacteria (*E. coli* or *P. syringae*) in soft agar (0.7% Agar) and poured into a plate containing hard agar (1.5% agar). A positive control consisting of *E. coli* host with a known concentration of bacteriophage and a negative control consisting of *E. coli* host with no bacteriophage were plated each time.

Study Design. We performed three cohort studies (A, B, and C), each with seven volunteers (male and female, 18–35 years old). Two (A and B) studied virus transfer from liquid-to-skin. The third (C) studied the transfer of virus from skin-to-liquid (Table 1). Written consent was obtained from all volunteers, and the experimental design was approved by the Research Ethics Committee of ETH Zurich. Volunteers were not asked to wash their hands or apply any antibacterial agent (such as alcohol-based disinfectant) prior to the experiment to mimic skin conditions likely encountered under natural conditions. Preparing hands by cleaning has previously been shown to significantly lower transfer efficiency of viruses between hands and surfaces.^{19,22} The hands of the volunteers were inspected before the experiment to ensure no skin damage. In studies A and B, the volunteers were asked to dip their fingers into a liquid containing bacteriophages (“finger immersion”). In study C, the volunteers’ hands were contaminated with bacteriophages and, subsequently, the bacteriophages were recovered from the skin using different liquids (“droplet transfer”).

Virus Transfer from Liquid-to-Skin. Virus transfer from liquid-to-skin was investigated as a function of virus concentration, virus species, liquid characteristics, and the contact time between the subject’s hand and the liquid solution. Cohort study A (seven subjects) was performed to investigate the influence of contact time and concentration on virus adsorption to the skin. Each one of the volunteers was assigned to one or two different bacteriophages (MS2, Q β or Φ 6). The volunteers were asked to come to the laboratory three times (three sessions), one for each concentration tested (10^6 , 10^7 , 10^8 PFU/mL), if one bacteriophage was assigned and 6 times if two bacteriophages were assigned.

A description of the experimental method with images can be found in the Supporting Information (SI) (Figure S1). Briefly, a circular area (diameter = 5 mm) was delimited in each finger of the volunteer using the rim of a 20 μ L pipet tip dipped in Vaseline (Vifor Pharma). We used Vaseline to delimit the circumference of the area sampled because of its hydrophobic properties and because virus adsorption to Vaseline is at least 1 order of magnitude lower than virus adsorption to the skin, as shown using MS2 (Supporting Information, S1.1). Subsequently, the volunteer was asked to dip ~5 cm of each finger into a flask containing a phosphate buffered saline solution (5 mM PO4–3 (Sigma-Aldrich) - 10 mM NaCl (Sigma-Aldrich) - pH 7.5 ± 0.1) with bacteriophage (MS2, Q β , or Φ 6) at different concentrations ($\sim 10^6$ – 10^8 PFU/mL). Each finger of the hand was immersed in the solution for a set period of time (between 5 and 1800 s) such that each hand could test five experimental conditions at a time. For each volunteer, one hand was the replicate of the other. Subsequently, the area inside the Vaseline was sampled. Sampling the area consisted of two consecutive rinsing steps. The first rinsing was performed pipetting up and down one time using the same phosphate

buffered saline solution, to remove the “unadsorbed bacteriophages”, and the second rinsing was performed pipetting up and down five times using a beef extract solution (3% beef extract (Sigma-Aldrich) - 0.1 M glycine (Fluka) - pH 8), to remove the “adsorbed bacteriophages”. Beef extract is a high ionic strength, high protein content solution used to desorb virus from surfaces.^{32,47} Every experiment had two negative controls, which consisted in sampling the area inside the Vaseline for the two fingers that were not used in the experiment. At the end of the experiment, subjects washed their hands with soap and disinfected with 70% ethanol.

Cohort study B was performed to investigate the effect of solution characteristics on virus transfer. We used the method previously described with some modifications: After application of Vaseline, the subjects were asked to dip the fingers into a flask containing different buffers with MS2 at a concentration of $\sim 10^7$ PFU/mL. To test the influence of liquid pH and ionic strength, six different buffers were used: 5 mM PO4–3–10 mM NaCl - pH 6 ± 0.1 , 5 mM PO4–3–550 mM NaCl - pH 6 ± 0.03 , 5 mM PO4–3–10 mM NaCl - pH 7.5 ± 0.07 , 5 mM PO4–3–550 mM NaCl - pH 7.5 ± 0.04 , 5 mM PO4–3–10 mM NaCl - pH 9 ± 0.03 , 5 mM PO4–3–550 mM NaCl - pH 9.1 ± 0.37 . Sixty seconds after the immersion of the fingers, the area inside the Vaseline was sampled as previously described.

Quantifying Virus Transfer from Skin-to-Liquid. Two scenarios of virus transfer from skin-to-liquid were evaluated: “Wet-Transfer” and “Dry-Transfer” (SI Figure S2). In both cases, a circular area (diameter = 5 mm) was delimited on the skin of the volunteer using Vaseline. Twenty microliters of saline buffer (5 mM PO4–3–10 mM NaCl - pH 7.5 ± 0.1) containing MS2 at a concentration of $\sim 10^7$ PFU/mL were pipetted onto the area inside the Vaseline (inoculum). The transfer occurred five seconds after inoculating the skin—when the droplet was still wet—or after the inoculum was visibly dried (between 15 and 30 min after inoculation). Transfer consisted of adding 20 μ L of Milli-Q water or 20 μ L of the volunteer’s saliva in the area inside the Vaseline. Immediately after addition, the full volume of the liquid was recovered. Subsequently, the skin was sampled by pipetting up and down five times using beef extract solution.

Statistical Analyses. The data were analyzed using the R statistical software (The R Foundation for Statistical Computing Platform, version 3.2.2). The significance of the difference in virus transfer between the different conditions was assessed using *n*-way ANOVA with Tukey’s post hoc comparisons for time and liquid conditions (pH and ionic strength) as well as for skin-to-liquid transfer. Linear regression models were used to assess the influence of bacteriophage concentration and virus species in virus transfer from liquid-to-skin. Statistical significance was assessed using a significance level of 0.05. All the analyses were conducted using the \log_{10} transformed data.

RESULTS

Virus Transfer from Liquid-to-Skin. A total of 210 transfer events in 27 sessions with seven volunteers were carried out. None of the negative controls showed bacteriophage contamination, implying the skin of the volunteers was free of the bacteriophages tested. Total virus transfer (adsorbed + unadsorbed) ranged from 3×10^3 to 4×10^6 PFU/cm² when concentrations of virus in the liquid varied between $\sim 10^6$ and $\sim 10^8$ PFU/cm³. The strongest predictor of virus transfer from liquid-to-skin was the concentration of bacteriophage in the liquid. Viral transfer scaled linearly with

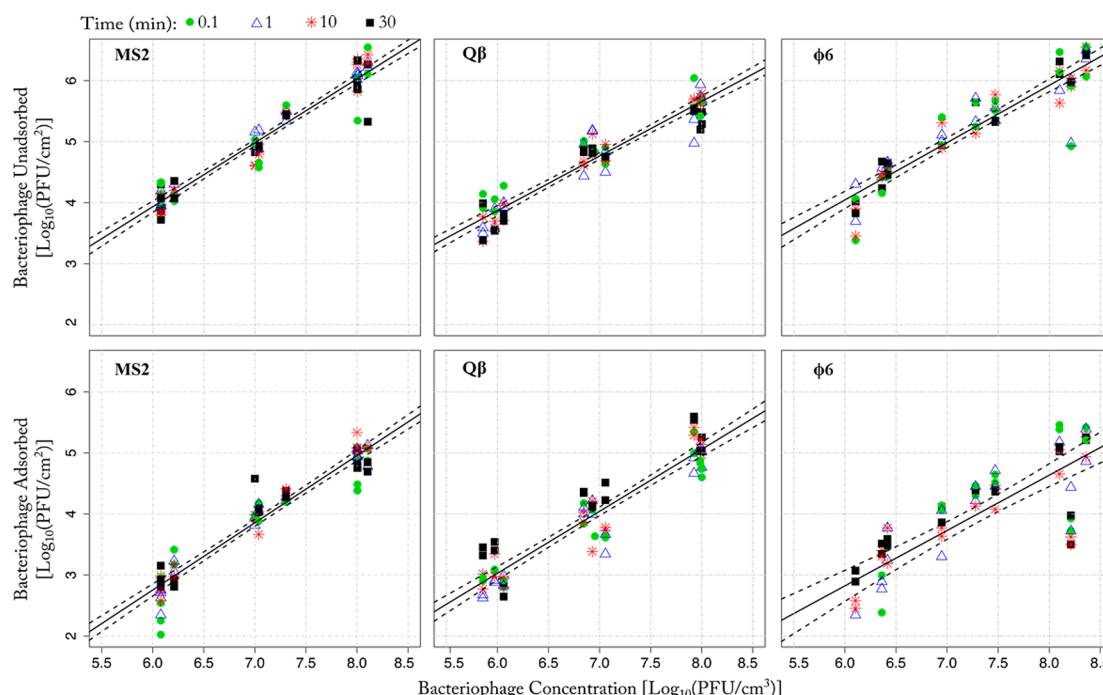


Figure 3. Linear regression of the log₁₀ transformed bacteriophage (MS2, Φ6, Qβ) transferred to the skin (adsorbed and unadsorbed) as a function of the log₁₀ transformed concentration of bacteriophage in the liquid. Four different contact times: 0.01 (green circle), 1 (blue triangle), 10 (red asterisk) and 30 min (black square) were evaluated. The 95% CIs for each regression line are shown in dotted lines. All figures share the same axes.

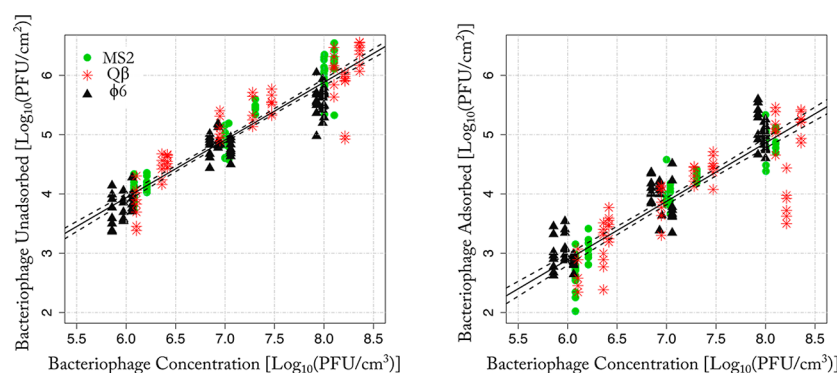


Figure 4. Linear regression for all bacteriophages (MS2, Qβ, and Φ6) transferred to the skin (adsorbed and unadsorbed) as a function their concentration in the liquid. The linear regression's 95% CIs are shown in dotted lines.

concentration (Figure 3). Using linear regression, concentration alone explained 88% of the variance in the unadsorbed (Linear Regression, $F(1, 209) = 1635$, $p < 0.001$) and 79% of the variance in the adsorbed ($F(1, 209) = 771.5$, $p < 0.001$) fraction of bacteriophages on the skin after a contact event.

When accounting for concentration, contact time between the liquid and the skin influenced the transfer of bacteriophage Qβ, but not MS2 nor Φ6. In the case of Qβ, there was a significant main effect of the time that the finger spent under the water on the unadsorbed fraction of virus transferred to the skin (two-way ANOVA, $F(3, 60) = 3.28$, $p = 0.03$, $\eta^2 = 0.14$) (SI Figure S3 and S4). Tukey post hoc test revealed that the amount of Qβ transferred when contact time was 30 min was statistically lower than 5 s ($p = 0.015$). We found a significant effect of contact time on the amount of virus adsorbed onto the skin ($F(3, 60) = 9.52$, $p > 0.001$, $\eta^2 = 0.32$). Tukey post hoc test revealed that the adsorption of Qβ when the contact time was 30 min was significantly higher than the adsorption at 5 s, 1 and 10 min ($p > 0.001$, $p > 0.001$, $p = 0.01$). Nevertheless, the

total transfer of Qβ -the adsorbed plus the unadsorbed fraction- was not influenced by contact time ($F(3, 60) = 1.31$, $p = 0.28$). In the case of bacteriophages MS2 and Φ6, we found no statistically significant difference in the unadsorbed fraction ($F(3, 59) = 0.46$, $p = 0.71$), ($F(3, 56) = 0.25$, $p = 0.86$) or in the adsorbed fraction ($F(3, 59) = 1.18$, $p = 0.32$), ($F(3, 56) = 1.18$, $p = 0.32$).

Bacteriophage species had a statistically significant influence on both the unadsorbed and the adsorbed fraction of bacteriophage transferred to the skin, but the effect size was small. Specifically, the contribution to the total variance explained by bacteriophage species was less than 2% (Figure 4). Additionally, liquid characteristics as described by liquid pH [6–9] and Ionic Strength [10–550 mM] did not significantly influence the unadsorbed ($p = 0.81$, $p = 0.70$) or the adsorbed ($p = 0.60$, $p = 0.44$) number of viruses transferred from liquid to the skin (Figure S5).

Modeling Liquid-to-Skin Transfer for Known Virus Concentrations. The data of virus transfer from liquid-to-skin

Table 2. Summary of the Mean Linear Model Parameters for the Log₁₀ Transformed Data-Slope (*m*) and Intercept (*b*)- with 95% CI, Number of Replicates (*N*) and the Goodness-of-Fit (*R*²) for the Unadsorbed and Adsorbed Fraction of the Three Different Bacteriophages^a

	unadsorbed				adsorbed			
	<i>m</i>	<i>b</i>	<i>N</i>	<i>R</i> ²	<i>m</i>	<i>b</i>	<i>N</i>	<i>R</i> ²
	(95% CI)	(95% CI)			(95% CI)	(95% CI)		
MS2 ^A	1.05 (1.11, 0.99)	−2.33 (−1.88, −2.79)	70	0.93	1.10 (1.02, 1.17)	−3.86 (−4.38, −3.33)	70	0.93
Qβ ^{AB}	0.90 (0.98, 0.86)	−1.55 (−1.13, −1.97)	71	0.91	1.01 (0.93, 1.10)	−3.06 (−3.68, −2.46)	71	0.89
Φ6 ^B	0.94 (1.01, 0.83)	−1.40 (−0.72, −2.07)	67	0.86	0.91 (0.74, 1.07)	−2.62 (−3.86, −1.38)	67	0.63
All	0.98 (1.02, 0.93)	−1.83 (−1.52, −2.14)	210	0.89	0.98 (0.91, 1.05)	−3.01 (−3.51, −2.51)	210	0.79

^aBacteriophages regression models with different letters (A, B) are significantly different (Multiple Regression).

Table 3. Summary of Skin-to-Liquid Theoretical Transfer Efficiency, Recoverable Transfer Efficiency and Total Recovery As a Function of Drying Condition and Liquid^a

	theoretical TE _{S-L} ^b mean ± SD [%]		recoverable TE _{S-L} ^c mean ± SD [%]		total recovery ^d mean ± SD [%]	
	wet transfer	dry transfer	wet transfer	dry transfer	wet transfer	dry transfer
skin-to-saliva	58.3 ± 14.8	20.1 ± 6.3	87.5 ± 6.3	44.4 ± 13.1	66.3 ± 15.6	49.5 ± 21.0
skin-to-water	89.7 ± 18.9	30.2 ± 16.7	95.0 ± 11.7	48.0 ± 14.3	94.1 ± 18.6	60.5 ± 21.6

^aThe mean and standard deviation for 14 replicates are reported. ^bTheoretical transfer efficiency= virus recovered in the liquid/(volume of the inoculum × concentration of virus in the inoculum) × 100. ^cRecoverable transfer efficiency= virus recovered in the liquid/(virus recovered in the liquid + virus recovered on the skin) × 100. ^dTotal recovery= virus recovered in the liquid + virus recovered on the skin × 100.

were adequately described by a linear regression model, as shown with *R*² values ranging from 0.63 to 0.93 for adsorbed or unadsorbed viruses (eq 6 and 7, Table 2). Specifically:

$$n = 10^b C^m \quad (6)$$

Which can be log-transformed:

$$\log_{10} n = m \log_{10} C + b \quad (7)$$

where *n* is the number of viruses transferred per unit surface area [viruses/cm²], *C* is the concentration of viruses in the liquid [viruses/cm³], and *m* and *b* are the slope and intercept estimated using linear regression of the log₁₀ transformed data. This regression model (eq 6) is equivalent to the Freundlich isotherm, which is a relationship commonly applied to virus adsorption.^{27,32,33,48–50}

Note that the constant *m* is not significantly different than unity in all but one case. Therefore, the eq 6 can be reduced to the linearized form *n* = 10^{*b*} *C*, which implies a linear relationship between both the adsorbed and the unadsorbed virus transferred, and the concentration of virus in the liquid.

Virus Transfer from Skin-to-Liquid. The theoretical transfer efficiency for skin-to-liquid (TE_{S-L}) was significantly influenced by the liquid used to recover the virus (saliva or water) (two-way ANOVA, *F*(1, 52)=26.8), *p* < 0.001, *η*² = 0.34). Skin-to-water transfer was, on average, 42% higher than skin-to-saliva transfer in wet conditions and 40% higher in dry conditions (Table 3). Allowing the liquid to dry on the skin before performing the transfer experiment significantly decreased the number of viruses transferred (*F*(1, 52) = 149.4, *p* < 0.001, *η*² = 0.74).

When the transfer efficiency was calculated using only the recovered virus (Recoverable TE_{S-L}), there was a small but significant effect of the liquid used to recover the virus on the percentage of virus transferred (*F* (1,52) = 4.7, *p* = 0.03, *η*²=0.08). In addition, the transfer was significantly lower when the liquid was dried prior the transfer event (*F* (1,52)=259.5, *p* < 0.001, *η*² = 0.83). Similarly, the total recovery was statistically significantly influenced by the liquid used (*F* (1,52) = 14.12, *p*

< 0.001, *η*² = 0.21) and allowing the inoculum to dry significantly decreased the total number of viruses recovered (*F* (1,52) = 23.88, *p* < 0.001, *η*² = 0.31).

DISCUSSION

Our study shows that transfer of viruses from liquid-to-skin can be described by a single model based only on the concentration of virus in the liquid. Therefore, this model applies to a wide range of water-related activities, including those that are short and long duration, with different liquids (for example, seawater, freshwater, urine), and with different viruses, including both enveloped and nonenveloped viruses. As shown in the present study, contact times considered (5 s to 30 min) had little-to-no influence on virus transfer, suggesting an apparent equilibrium between viruses adsorbed on the surface and virus in the liquid was reached within five seconds. Similarly, there was no statistically significant difference observed between the liquid conditions (pH 6–9, IS 10–550 mM) and the differences observed between viruses (MS2, Qβ, and Φ6) tested were not biologically meaningful as defined here by having an effect size of less than 2%. This differs from previous studies showing virus species and liquid conditions influence virus adsorption to surfaces in a variety of batch and flow-through column experiments^{26,27,51} as well as Quartz Crystal Microbalance with Dissipation (QCM-D) experiments.^{29,30}

The apparent discrepancy between our cohort studies and previous liquid-to-surfaces studies is likely attributed to differences in experimental methods and natural heterogeneity of skin surfaces. The three cohort studies were performed at virus concentrations between 10⁶ and 10⁸ PFU/ml. Higher concentrations were considered not relevant for most exposure assessments, while lower concentrations resulted in transfer quantities close to our limit of detection (100 PFU/cm²). Additionally, we asked the volunteers not to wash their hands prior the experiment, potentially leading to a high between-subjects variation, as compared with within-subjects variation. This could have obscured the contribution of other factors (virus species, contact time, liquid characteristics) to virus

transfer. We did not collect data on skin characteristics (e.g., pH, water content) or personal hygiene information (e.g., time since last hand washing). The study was insufficiently powered to assess these factors, though they may have influenced transfer.

The transfer model from liquid-to-skin highlights the importance of the volume of liquid remaining on the skin after contact and the concentration of viruses in the liquid. Water volume influences the number of “unadsorbed” viruses that are transferred to the skin, whereas concentration influences both “unadsorbed” and “adsorbed” fraction of viruses. This result is consistent with studies of virus transfer between skin and fomites¹⁹ where the concentration of viruses on the fomites was proportional to the number of viruses transferred to the skin. In the present study, the “unadsorbed” fraction was the main driver of virus transfer. After liquid-to-skin contact, removing the excess liquid on skin will significantly reduce the indirect (water-mediated) transfer of viruses, but a fraction of the viruses will remain on the skin (“adsorbed”) even after complete removal of the liquid.

In addition to our liquid-to-skin model, we developed a model for subsequent skin-to-liquid transfer. The wetness of the skin significantly influences virus transfer. Up to $90 \pm 19\%$ of the virus inoculated on the skin are ready to be transferred to the water when the inoculum is wet and $30 \pm 17\%$ when it is dry. The relatively low transfer of viruses in dry conditions could be partially explained by the inactivation of nonenveloped viruses on human hands. Ansari et al. (1988) estimated that only 57% and 43% of rotavirus was recoverable 20 and 60 min after inoculation onto hands, respectively.³⁶ Skin-to-saliva transfer was significantly less than skin-to-water, which can be partially explained by saliva’s antiviral properties.^{52–54} Our experimental controls showed that MS2 counts were 19% lower if the MS2 was kept for 2 to 6 h in PBS + saliva as compared with PBS + water (SI S3.1). Our work on virus transfer from skin-to-saliva provides insight into virus transfer during hand-to-mouth contacts, which contribute to infection risks^{15,17,55–57} and are relatively frequent, especially among children.^{58,59}

The enveloped bacteriophage $\Phi 6$ behaved similarly to the two nonenveloped viruses. Most studies of virus fate in the environment are based on nonenveloped viruses since they are more persistent than enveloped viruses. Nevertheless, it has been shown that enveloped viruses can survive for days to weeks, depending on virus characteristics and environmental conditions.^{38,39,44,60,61} Furthermore, in an aqueous environment, enveloped viruses are readily adsorbed onto the solid phase.³⁹ The risk that potentially pandemic enveloped viruses such as ebola, avian influenza, Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS) pose for human health⁶² highlights the importance of studying the fate and transfer of enveloped viruses. Our data suggest that when modeling virus transfer between liquid and skin, the same model can be used for enveloped and nonenveloped viruses. Although transfer efficiency may be similar, risks may still differ for enveloped viruses due to differences in transmission route (i.e., bloodborne transmission as opposed to fecal-oral transmission).

Our results suggest that consideration of virus transfer (from liquid-to-skin) due to bulk liquid transfer alone underestimates infection risks. To date, the virus adsorption component has been neglected in risk assessments. As previously discussed, De Man et al. used information on the thickness of the water film that remained on the skin and the pathogen concentration in

the water to estimate the total pathogen transfer.¹⁵ Using their assumptions (average film thickness = 2.16×10^{-3} cm), when concentration is 10^4 viruses/cm³, a total of 21.6 viruses/cm² would be transferred to the skin, which represents the “unadsorbed” fraction. Based on our model (eq 5), the number of viruses adsorbed to the skin when virus concentration is 10^4 viruses/cm³ is equal to $10^{-3.01} \times 10^4$, which is 9.8 viruses/cm². These results would shift the estimate 37%, from 21.6 to 31.3 ($21.6 + 9.8$) viruses/cm². De Man et al. assumed that 100% of the unadsorbed pathogens are transferred to the mouth after contact (21.6 pathogens/cm²). Our results suggest that, in the case of viruses, only 58.3% are transferred after hand-to-mouth contact, when the hand is still wet, leading to a final transfer of 18.3 viruses/cm² ($31.3 \text{ viruses/cm}^2 \times 0.58$).

Indirect transmission of pathogens is especially important in scenarios involving children playing in areas exposed to liquids contaminated with viruses such as slum flooded zones⁶³ and open drains,⁶⁴ as hand-to-mouth contact frequency in children is high.⁵⁸ Future QMRA studies should incorporate indirect transmission of pathogens through water to evaluate its relative contribution to overall risk. Transfer from liquid to skin should be modeled to quantify the number of viruses that remain transferable during subsequent hand-mouth contacts, which includes the number of viruses adsorbed on the skin and the number of viruses retained in the liquid on the skin. The volume of liquid remaining on skin depends on the activity performed.⁵⁸ In this work we suggest an equation to estimate the unadsorbed fraction of viruses as a function of concentration, which could be used in QMRA studies if no prior information regarding the amount of liquid transferred is known. Nevertheless, the volume of liquid retained on the skin in the experiments performed could have been influenced by the experimental method, therefore, a more comprehensive study of liquid transfer to skin after different activities is needed to have a more accurate estimate of the number of unadsorbed viruses on the skin.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04949.

A figure of the method used to estimate virus transfer from (1) liquid-to-skin and (2) from skin-to-liquid; a description of the adsorption of bacteriophage to Vaseline compared to human skin; a description of the influence of the droplet environment on bacteriophage MS2 survival; a figure of bacteriophages (MS2, $Q\beta$, and $\Phi 6$) transferred to the skin as a function of contact time for three different concentrations; a figure of bacteriophage $Q\beta$ transferred to the skin as a function of concentration and contact time; a figure of the adsorption of bacteriophage MS2 to the skin as a function of liquid characteristics; a description of bacteriophage MS2 survival in saliva and water (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was funded by Eawag Discretionary Funds for Research. We thank the volunteers for their participation.

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